Southeastern Cooperative Wildlife Disease Study
College of Veterinary Medicine
University of Georgia
589 DW Brooks Drive
Athens, Georgia 30602
TEL 706-542-1741 | FAX 706-542-5865

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TO: Ben Robinson, Director of Wildlife Division
Kentucky Department of Fisheries and Wildlife Resources
Frankfort, Kentucky

Joe Benedict, Chief of Wildlife & Forestry Division
Tennessee Wildlife Resources Agency
Nashville, Tennessee

FROM: Mark G. Ruder, DVM, PhD, Director
Ellen Haynes, DVM, PhD, SEAFWA Regional Wildlife Health Coordinator
Southeastern Cooperative Wildlife Disease Study

RE: CWD Diagnostic Test Interpretation

Dear Ben and Joe:

At the request of the Kentucky Department of Fisheries and Wildlife Resources and the Tennessee Wildlife Resources Agency (TWRA), SCWDS is providing guidance on how agencies interpret and respond to inconclusive or conflicting chronic wasting disease (CWD) diagnostic test results. The specific concern centers on tissue samples from deer that repeatedly test positive (referred to as initial reactors) using a screening test (i.e., enzyme linked immunosorbent assay; ELISA) but are not confirmed using the standard confirmatory test (i.e., immunohistochemistry; IHC), either because the IHC result was *not detected* or because it was not run on the sample. Studies have found that IHC will yield *not detected* results for a small percentage (~5-6%) of samples that test as initial reactors using the ELISA, which effectively creates a diagnostic gray zone. Although such a gray zone can be expected with any diagnostic test, this uncertainty can be difficult to navigate with a disease like CWD, for which positive diagnostic test results can carry significant implications for deer populations, wildlife managers, and the public. Such results in an individual deer are difficult, if not impossible, to resolve and may ultimately mean that the animal was truly infected with CWD, or that it falsely tested positive. In these instances, especially **in areas where CWD has not been documented, additional information from the population is needed and we recommend agencies increase surveillance in the area to gain clarity.**

Additional Information

In general, all diagnostic tests have inherent limitations. These limitations may be related to sample collection and quality, what the test is detecting, the ability of the test to detect its target (i.e., test sensitivity), and the ability of the test to differentiate between its target and similar non-targets (i.e., test specificity). Diagnostic tests are often used in combination to help overcome limitations of individual tests and gain overall confidence in the diagnostic testing process. In all cases, results must be

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interpreted based on the limitations of the test(s), what is known about the animal and population being tested, and the implications of particular results.

Currently, the United States Department of Agriculture (USDA) officially recognizes two routinely utilized CWD diagnostic tests for use in deer: ELISA and IHC. All CWD surveillance programs conducted by wildlife agencies currently depend on one or both of these two diagnostic test platforms. The ELISA test is sensitive, rapid, and readily available in many laboratories and is used as an initial screening test to address the large number of samples (often thousands per state) submitted to diagnostic laboratories as part of CWD surveillance programs. The ELISA results are reported as "not detected" or "suspect positive;" the latter are also termed initial reactors. The IHC test is slightly more difficult, expensive, and time-consuming to perform but is considered by USDA to be the "gold standard" test and is often used as a "confirmatory" CWD test. Specifically, when the tests are used in tandem, samples that are initial reactors (i.e., "suspect positive") by the ELISA test are confirmed as positive when they also test positive by IHC. Typically, IHC results are accepted as final, whether the result is "positive" or "not detected". The agreement between ELISA and IHC tests is typically very good and most often the samples that are initial reactors by ELISA are confirmed as positive by the IHC test. When a sample tests as an initial reactor by ELISA, it is common laboratory practice to repeat the test at least two more times before reporting, to gain confidence in the result. However, a small percentage (~5-6%) of samples may repeatedly test as initial reactors by the ELISA test but yield a *not detected* IHC result and such samples are typically interpreted as "CWD not detected." As of August 2022, TWRA now reports samples from outside of existing CWD management areas with this discrepancy as "Suspect- Not Confirmed."

Since ELISA results have been shown to be reliable and have a high level of agreement with IHC results, wildlife agencies will often choose to rely upon ELISA results without IHC confirmation in areas where CWD is established in a deer population (i.e., endemic). This approach saves valuable resources for agencies. Positive CWD test results from samples from areas where CWD is *not* known to be present typically have greater management implications; therefore, when testing animals from these areas, it is common for agencies to consider initial reactors on ELISA tests as CWD-positive animals only if they are confirmed with the IHC test.

When a sample is repeatedly an initial reactor by the ELISA test, but the IHC result is *not detected*, questions may arise about the true infection status of that animal. Unfortunately, additional scrutiny of such samples often does little to reduce the uncertainty surrounding that particular animal. Based on the epidemiology of CWD, if the deer in question was truly infected, there are likely to be additional CWD-positive animals in that population that can be detected through surveillance programs. Therefore, additional surveillance testing of other deer from the same area is warranted to determine if CWD is present in that area.

Based on the current knowledge surrounding CWD and the diagnostic gray zone possible with the available USDA-approved diagnostic tests, SCWDS encourages state agencies to, when possible, invest agency resources to increase surveillance efforts in areas with inconclusive CWD test results (i.e., initial reactor ELISA results that are not confirmed with IHC), particularly in areas where CWD has not previously been reported.